ANALYSES OF CLASS AND SUBCLASS ANTIBODY OF CIRCULATING IMMUNE COMPLEXES IN CHILDREN WITH SEVERE *PLASMODIUM FALCIPARUM* MALARIA IN ENDEMIC REGIONS OF WESTERN KENYA

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*Plasmodium falciparum* infection is characterized by deadly complications such as severe malaria-associated anaemia (SMA) and cerebral malaria (CM). The exact mechanisms underlying pathogenesis of these severe forms of *Plasmodium falciparum* malaria are not fully understood yet they are associated with a lot of morbidity and mortality. Studies have shown a link between severe *P. falciparum* malaria and levels of circulating immune complexes (CIC) but the exact role of these CICs in the pathogenesis of severe *P. falciparum* malaria is still unclear. This study aimed to investigate the quantitative and qualitative differences in antibody classes and subclasses in serum immune complexes (ICs) between children with the severe forms of *P. falciparum* malaria and those with uncomplicated malaria as well as identifying the predominant *P. falciparum* antigens that contribute to IC formation in these clinical groups. A total of 75 children with SMA and 32 children with CM were enrolled from hospitals in western Kenya and matched with 74 and 52 control children respectively with uncomplicated symptomatic malaria. IC levels were measured using solid phase ELISA protocols and antibody classes and subclasses were identified using polyclonal sera for the classes or monoclonal antibodies for the subclasses. ICs were purified using polyethylene glycol (PEG) precipitation. The isolated ICs were dissociated by using an acidic buffer (Glycine-HCL pH 2.0). These were then electrophoresed on one-dimensional and two-dimensional polyacrylamide gel blotted by Western transfer and probed using human anti-*P. falciparum* antibodies. The study showed a general increase in levels of ICs as a result of *P. falciparum* infection in severe malaria cases and their symptomatic controls. Although IgG IC levels were elevated in children with severe malaria upon enrolment, children with CM had the highest levels of ICs for all the antibody classes. Conditional logistic regression showed a borderline association between IgG4-containing ICs and increased risk of SMA (OR = 3.11, 95% CI 1.01 to 9.56, P = 0.05). Total IgG-containing ICs (OR = 2.58, 95% CI 1.20 to 5.53, P < 0.02) and IgE-containing ICs (OR = 3.27, OR 1.38 to 7.78, P < 0.01) were associated with increased risk of CM. Six specific *P. falciparum* antigens were found to be associated with severe malarial anaemia while another three antigens were associated with cerebral malaria when compared to their specific controls. While when SA and CM where compared together, a 91Kda antigen was highly associated with SA (P < 0.01), while a slightly lighter antigen of about 87 Kda was significantly associated with CM (P < 0.01). These findings have demonstrated quantitative and qualitative differences in ICs in children with SMA and CM and this underscores the potential mechanisms of the pathophysiology of the disease. Furthermore the findings of this study suggested having higher IgG4-containing ICs is a risk factor for SMA while higher IgG and also IgE-containing ICs are both associated with CM pathology. This suggest that although SMA and CM were characterized by high levels of ICs, the class and
subclass make up of these ICs as well as the role that they play in each may be distinct. This study demonstrated an association between malaria antigens and severity of the disease hence there is need for full characterization of the parasite antigens. These findings may contribute to a better understanding of the role of different antibody classes and subclasses in protective or damaging mechanisms and may provide new insights into development of effective malaria control strategies and vaccine development.